

Reproduction in two sympatric Iberian nudibranch species (Gastropoda: Opisthobranchia) with contrasting development types

Reproducción en dos especies simpátricas de nudibranquios (Gastropoda: Opisthobranchia) de la Península Ibérica con tipos de desarrollo alternativos

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ABSTRACT

Doriopsilla areolata and *D. pelseeneri* are two sympatric species from the Iberian Peninsula that contrast in their reproductive modes. In this study we investigated the developmental mode of both species, comparing egg number and egg volume. In addition, we investigate whether the type of development influences the amount of energy allocated for reproduction.

Animal weight was found to be positively correlated with egg number per egg mass but not with egg volume, which seems to be a specific character in both species. Moreover, our results suggest that both species allocate the same amount of energy for reproduction, in terms of body weight, though a clear difference in the way these resources are used was actually observed. *Doriopsilla areolata* invested $2.22 \pm 1.87\%$ of body weight ($n=36$) essentially for egg production, whereas *D. pelseeneri* invested $5.72 \pm 3.31\%$ of body weight ($n=11$) on rich gel matrix spawn masses. The different type of development could provide an explanation for the difference in the geographical range of both species, but further studies are required.

RESUMEN

Doriopsilla areolata y *D. pelseeneri* son dos especies simpátricas en la Península Ibérica, que contrastan en sus modos de reproducción. En este trabajo se investigó el modo de desarrollo de ambas especies, comparando el número y el volumen de huevos. Además, se investiga si el tipo de desarrollo influye en la cantidad de energía dedicada a la reproducción.

El peso del animal muestra una correlación positiva con el número de huevos por masa de huevos, pero no con el volumen del huevo, que parece ser un carácter específico en ambas especies. Nuestros resultados sugieren que ambas especies asignan, en términos de peso corporal, la misma cantidad de energía para la reproducción, pero la forma en que estos recursos se utilizan es diferente. *Doriopsilla areolata*, invirtió $2,22 \pm 1,87\%$ de su peso corporal ($n = 36$), esencialmente para la producción de huevos, mientras que *D. pelseeneri* ha invertido $5,72 \pm 3,31\%$ de su peso corporal ($n = 11$) en la matriz de gel enriquecido de las masas. El tipo de desarrollo podría explicar la diferencia en la distribución geográfica de las dos especies, pero se necesitan más estudios.

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INTRODUCTION

As far as their reproduction is concerned, opisthobranchs display a diversity of strategies (RUDMAN AND WILLAN, 1998). Several works report on species displaying different larval development types: planktotrophy, lecithotrophy, and direct development (HADFIELD AND SWITZER-DUNLAP, 1984; HADFIELD AND MILLER, 1987; BOUCHET, 1989; TODD, 1983). However, the selective factors in determining these larval strategies remain to be ascertained. According to HADFIELD AND MILLER (1987), for any of those three "different means to the same end" the time required to reach the juvenile stage is variable; usually, the larger the egg, the longer the pre-hatching period.

Doriopsilla areolata (Bergh, 1880) and *Doriopsilla pelseeneeri* (d'Oliveira, 1895) are two radula-less, porostomate nudibranchs belonging to the family Dendrodorididae. They are amongst the most common nudibranchs inhabiting the Iberian coasts. Although sympatric and occasionally feeding upon the same sponges (GC, personal observations), both species have rather different types of development. *Doriopsilla areolata* has a planktotrophic development type, producing egg masses that are formed by numerous small eggs, while *D. pelseeneeri* displays a metamorphic, direct development type and produces a lower number of larger eggs (SOARES AND CALADO, 2006).

The known geographical range is also different. *Doriopsilla areolata* is known to occur between the North coast of Spain and the Cape Verde Islands (including the Mediterranean Sea), Ghana and Angola, whereas *D. pelseeneeri* is restricted to the Atlantic and Mediterranean coasts of the Iberian Peninsula. The aim of this study is to compare the developmental mode of both species, comparing egg number and egg volume. Further, we intend to test whether the type of development influences the amount of energy allocated for reproduction.

MATERIALS AND METHODS

Animals of both species were collected from two localities along the Arrabida coast (West Portuguese Coast) and Ria de Ferrol (Galicia, NW Spain). The exact localities can be found in SOARES AND CALADO (2006). All specimens were collected by hand while scuba diving at 5-15 m depth from March to July of 2003, in the period reported as the peak of their reproduction period.

Animals were maintained under laboratory conditions as described elsewhere (SOARES AND CALADO, 2006). Specimens were paired periodically for copulation and after approximately one week, egg masses were laid and removed immediately. In order to estimate the number of eggs, spawn masses were measured (top, base length, and width). We collected a total number of 61 *Doriopsilla areolata* and 39 *D. pelseeneeri*, from which we studied 43 and 21 laid egg masses, respectively. Each set animal-spawn mass was weighed in fresh for later use in calculations.

As for *Doriopsilla areolata*, three 1mm wide transverse sections of the egg masses were cut for observation under a stereomicroscope. Spawns from *D. pelseeneeri* were observed intact under a stereomicroscope. Immediately after oviposition, and before any cleavage was observed, eggs were counted in a known egg mass area and its larger diameter measured to estimate egg number per egg mass and egg volume, respectively.

In order to establish a calibration curve fresh weight/dry weight, three *Doriopsilla pelseeneeri* specimens and four *D. areolata* specimens were weighed without water excess and dried at 60°C to constant weight (SMITH AND SEBENS, 1983). Afterwards, a fraction of all dried samples were used to calculate ash values by ignition (3 hours at 550°C in a muffle furnace), by the estimation of ash-free dry weight equivalents (HAVENHAND AND TODD, 1988; TODD, 1979). The same procedure was used on five egg masses of each species, as well

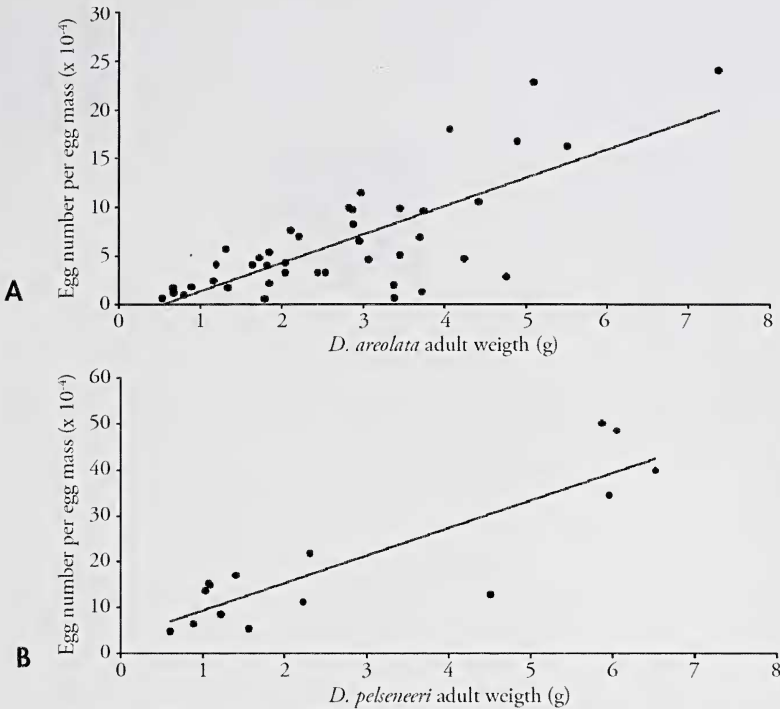


Figure 1. Relationship between egg number per egg mass and adult animal weight for (A) *D. areolata* ($R^2= 0.563$) and (B) *D. pelseneeri* ($R^2= 0.768$).

Figura 1. Relación entre el número de huevos por masa de huevo y peso del animal adulto en (A) *D. areolata* ($R^2= 0,563$) y (B) *D. pelseneeri* ($R^2= 0,768$).

as in two spawn masses of *D. pelseneeri*, from which eggs were previously removed manually. For *D. areolata* it was assumed that egg masses were entirely composed of eggs, since it was impossible to detach eggs from the feeble gel matrix. A measure of the reproductive effort per egg mass was calculated as the ratio between the dry weight of a single egg mass and the dry weight of the progenitor adult inferred from the fresh weight by the calibration curve. Finally, how much energy (inferred by the amount of biomass) is allocated per egg mass and per egg unit, was calculated.

Statistical analyses were conducted using STATISTICA (Release 6.0). Regression analysis was performed to investigate the relationship between both egg number and egg volume (mean values) and adult body weight. Differences

between egg masses of the distinct populations (Portugal and Spain) were also investigated.

RESULTS

Egg number per egg mass and egg volume

As presented in SOARES AND CALADO (2006), the egg number per egg mass in *D. areolata* was estimated to be between 5,500 – 240,000 eggs ($n=43$), whereas in *D. pelseneeri* egg number varied from 300 to 5,000 ($n=15$) eggs per egg mass.

In both species it is clear that egg number in a single spawn mass is positively correlated with animal weight ($R^2= 0.563$ for *Doriopsilla areolata* and $R^2= 0.768$ for *D. pelseneeri*) as can be seen in Figure 1. The geographical area

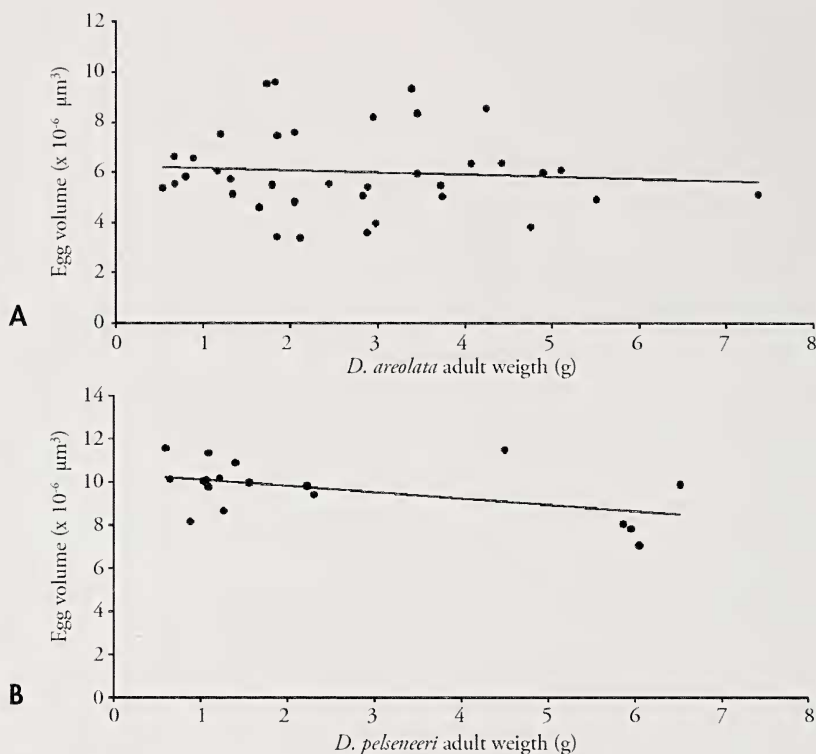


Figure 2. Relationship between egg volume and adult animal weight for (A) *D. areolata* ($R^2=0.006$) and (B) *D. pelseneeri* ($R^2=0.240$).

Figura 2. Relación entre el volumen del huevo y peso del animal adulto en (A) *D. areolata* ($R^2=0,006$) y (B) *D. pelseneeri* ($R^2=0,240$).

from where animals were collected seems to have no influence on this analysis. However, specimens from Galicia were observed to be slightly smaller and therefore to deposit smaller egg masses than those collected in Portugal.

Moreover, it was observed that egg volume does not appear to co-vary with animal weight ($R^2=0.006$ for *Doriopsilla areolata* and $R^2=0.240$ for *D. pelseneeri*) as illustrated in Figure 2. Indeed, egg volume seems to be a specific character in both species.

Reproductive investment in the two species

Dry weight analysis revealed that *Doriopsilla pelseneeri*'s body contains on average $87.94 \pm 2.22\%$ ($n=3$) of water,

whereas *D. areolata* contains $84.20 \pm 2.41\%$ ($n=4$), which reveals a similar hydrate condition in both species. From the ash content analysis it was estimated that *D. pelseneeri* contains $4.30 \pm 1.42\%$ ($n=3$) of inorganic matter in the body, whereas *D. areolata* contains $7.95 \pm 1.34\%$ ($n=4$) (mean values).

Doriopsilla pelseneeri egg masses contained $90.57 \pm 3.03\%$ ($n=5$) of water and $4.13 \pm 0.89\%$ ($n=5$) of inorganic matter, whereas *D. areolata* egg masses contained $92.73 \pm 2.08\%$ ($n=5$) of water and $4.55 \pm 0.85\%$ ($n=2$) of inorganic matter (mean values). In the former species egg masses are made of 39% of eggs and 61% of gel matrix, which contains $88.53 \pm 1.66\%$ ($n=2$) of water and $6.90 \pm 1.08\%$ ($n=2$) of inorganic matter. Since for *D. areolata* it has been impossible to detach

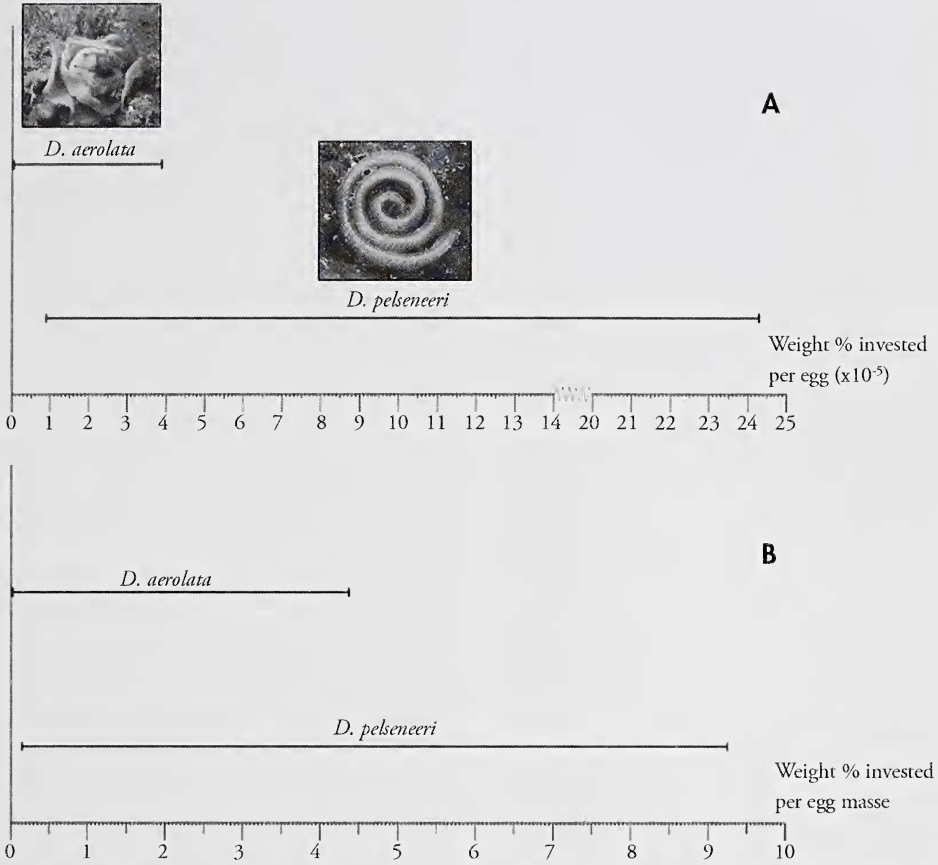


Figure 3. Relationship between weight invested (A) per egg mass and (B) per egg (%) for *D. areolata* and *D. pelseneeri*.

Figura 3. Relación entre el peso invertido (A) por unidad de masa de huevos y (B) por huevo (%) para *D. areolata* y *D. pelseneeri*.

eggs from the jelly matrix, egg masses were assumed to be made up nearly 100% of eggs.

With these values we obtain that *D. pelseneeri* invested $0.0012 \pm 0.0009\%$ of their weight per single egg ($n=11$, mean value of the range $0.00009\% - 0.00243\%$), while *D. areolata* invested $0.00026 \pm 0.00009\%$ of their body weight per single egg ($n=36$, mean value of the range $0.0000032\% - 0.00039\%$) (Figure 3A). The investment per egg mass was also calculated; *Doriopsilla pelseneeri* specimens studied invested $5.72 \pm 3.31\%$ of their body weight ($n=11$, mean value of the range $0.305\% - 9.479\%$), whereas

D. areolata, invested $2.22 \pm 1.87\%$ ($n=36$, mean value of the range $0.012\% - 4.767\%$) (Fig. 3B).

DISCUSSION

Developmental patterns in opisthobranchs have been a subject of much interest in the last decades (e.g. HADFIELD AND SWITZER-DUNLAP, 1984; HADFIELD AND MILLER, 1987; TODD, 2001, KRUG, 2009). However, obtaining a final consensus has not been possible so far and some authors claim that there is no single answer to what determines the

developmental mode of any individual opisthobranch species (HADFIELD AND MILLER, 1987). Although sympatric and feeding on the same food items, *Doriopsilla pelseneeri* and *D. areolata* exhibit different developmental patterns. SOARES AND CALADO (2006) demonstrated that *D. areolata* with planktotrophic development takes approximately half the time till hatching than *D. pelseneeri* with direct development.

Even with rather opposite developmental modes both species allocate about the same amount of energy for reproduction, as inferred from differences in body weight. However, there is a clear difference in the way these resources are used. *Doriopsilla areolata* expends most resources producing spawns with a high number of small eggs, and a very scarce gel matrix. Larvae hatch just a few days after egg mass deposition, as is typical for obligatory planktotrophs, and thus not many nutritive reserves are needed. In opposition, *D. pelseneeri* produces a small number of large, yolky eggs and a dense gel matrix in which they are embedded. The matrix is used for mechanical and, most certainly, for chemical protection of the embryos developing inside the egg-capsule. Since we did not have access to the complete reproductive period, we were unable to calculate the total reproductive effort for these two species and therefore to compare it with others (e.g. TODD, 1979; DEFRESE AND CLARK, 1983; GIBSON AND CHIA, 1991).

Another conclusion arises from our results, larger adult specimens produce higher numbers of eggs per egg mass. This has been observed for both species studied herein. ALLEN, KRUG AND MARSHALL (2009) report for the sacoglossan *Elysia stylifera* that larval size is determined by the extra-capsular yolk, but not by egg size. TODD, LAMBERT AND DAVIES (2001), based on previous studies with *Adalaria proxima* (Alder and Hancock, 1854) (LAMBERT, TODD AND THORPE, 2000) generalise that between-population variation in egg size is genetically determined. Such a pattern was not observed herein. Egg size appears to be species

specific regardless of the geographic area, and hence of the population.

As STRATHMANN (1986) refers, "differences in size and body plan account for much of the variation in types of larval development". Recently and using opisthobranchs as a model, GODDARD (2004) agrees with this point of view. This author argues that phylogenetic constraints on developmental mode can be severe. For instance, if a feeding structure is lost the possibility of it being regained is very remote, constraining the whole lineage to a non-feeding developmental mode. In gastropods, however, this process could have happened more easily since lecithotrophic larvae often retain structures used in the process of feeding (STRATHMANN, 1978).

Since planktotrophy is clearly the most frequently observed development mode, HADFIELD AND MILLER (1987) assume that this is also the most primitive developmental strategy amongst opisthobranchs. They argue that the evolutionary direction will be towards lecithotrophic-planktonic development and from there to direct development, in which the opisthobranchs with ameta-morphic development are the most evolved forms. According to TODD (2001), a larger egg should not be the trigger for this change and for HADFIELD AND MILLER (1987) it is not clear if the selective pressures responsible for this have been the same in all opisthobranch species. Intense predation on early juveniles could have selected for increased size, leading to a direct development mode, but in another situation the opisthobranch can be the predator and may adapt its own life cycle to grow and reproduce before a short-living prey is exhausted (TODD AND DOYLE, 1981). The costs for this adaptation will be, in part, a reduction in opisthobranch size and fecundity, and production of larger, lecithotrophic eggs or directly developed offspring (pelagic larva could be dispensable if prey is not too patchy) will be favoured (HADFIELD AND MILLER, 1987). According to TODD (1991) a consensus is lacking and probably

there are special cases for particular species.

Planktotrophic species have a high fecundity and great dispersal potential, whereas species with direct development have a reduced fecundity with a higher larval stage protection and usually hatch and settle in the same places where their progenitors live (SCHELTEMA, 1986). The data available on the two species herein studied support this idea. *Doriopsilla areolata*, with planktotrophic development, has a wide geographic range whereas *D. pelse-neeri*, with direct development, has a much more restricted distribution, mostly limited to the Iberian Peninsula (see also VALDÉS AND ORTEA, 1997). Potential dispersion capability of a species is dependent on many physical and biological habitat conditions (SCHELTEMA, 1986), however, and since both species feed on a variety of sponges widespread in the Lusitanian province (GC, personal observations), no host constraint should be involved in their actual distribution.

Studying two sympatric dorid nudibranchs with different larval strategies, TODD (1979) claimed that planktotrophic species were "forced" into that developmental mode since an insufficient number of lecithotrophic eggs would be produced to maintain its population stability. This author also suggested that selection always favours the strategy

which produces the larger offspring survivorship even if it is energetically inefficient. However, as seen above, other authors claim that energetic factors seems to be a lesser deterministic factor for the selection of developmental pattern.

As HADFIELD AND MILLER (1987) already pointed out, there is "no valid reason to assume that species currently found together evolved in sympatry or under the conditions in which they are now found". Furthermore, it is not certain that planktotrophy and lecithotrophy are different processes aiming at the same final results (TODD, 1991). We are convinced that further phylogeographic studies on the populations of *Doriopsilla areolata* and *Doriopsilla pelse-neeri* will help to elucidate the origins of the speciation process and therefore the evolutionary relationships between these two species.

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